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Evaluation of family growth response to fishmeal and gluten-based diets in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

A study was conducted to evaluate genotype × feed interactions in a commercial strain of rainbow trout (*Oncorhynchus mykiss*). Microsatellite DNA markers were used to determine the pedigree of the top 1% and bottom 1% of progeny in a large scale commercial growth trial of 24,000 rainbow trout from 20 full-sib families (20 dams × 10 sires in a nested mating design). The progeny were pooled at eyed stage and divided into 2 groups. Half of the fish from each family were fed a standard fishmeal-based diet and the other half was fed a plant protein (gluten)-based diet to determine the relative family rankings in each diet. The primary protein sources in the plant protein-based diet were corn gluten and wheat gluten meals. Krill was supplemented to this feed for the early life stages (starter, #1, #2, #3 crumbles), but was eliminated in the larger pellet sizes. Large genetic variation for growth was identified for both diets and the sire effect was found to be highly significant ($P<0.001$). The family rankings were similar for both diets, which suggest that the fish that grow faster on fishmeal diet are likely to grow faster on plant protein-based diets, and therefore current commercial strains that exhibit superior growth should retain their improved performance if raised on gluten-based diets. Multiplexing microsatellite markers would further improve the efficiency of parentage assignment protocols in large-scale rainbow trout selection programs.
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Keywords: *Oncorhynchus mykiss*; Selection; Plant diets; Breeding; Microsatellites; Parentage determination; Triploidy

1. Introduction

The aquaculture industry has received a large amount of criticism in recent years regarding the volumes of fishmeal and fish oils used in the manufacture of feeds, particularly for salmonid diets. Increasing concerns over potential negative environmental impacts, from both an effluent water quality standpoint

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and the use of wild-caught fish as feed ingredients (Goldberg et al., 2002), have prompted increased examination of alternate diet formulations for aquaculture (Gomes et al., 1995; Hardy, 1996; Sugiura et al., 1999; Carter and Hauler, 2000; Kissil et al., 2000). The anticipated changes in feed formulations have raised concerns regarding the ability of fish selected for rapid growth on traditional, fishmeal-based diets to effectively utilize these alternate diets (Blanc, 2002).

Genetic improvements in aquaculture species have been reported with increasing frequency in recent years (Gjedrem, 2000). Results from commercial rainbow trout breeding programs have shown gains of approximately 15% per generation in selections for body size (James Parsons, unpublished data). Many aquaculture selection programs utilize family-based mating design and benefit from the high fecundity of most aquatic species, external fertilization which enables simultaneous multiple matings and the use of semen storage and cryopreservation for delayed fertilization. Full- and half-sib families possess the appropriate genetic relationships for estimating breeding values and genetic correlations among traits of interest (Falconer and Mackay, 1996). However, difficulty in marking small aquatic species has often necessitated the rearing of early developmental stages in individual family tanks, resulting in shared tank effects by family members that were reared together (Winkelman and Peterson, 1994). Additionally, performance when the families are reared separately is not necessarily representative of the performance in mixed family tanks (Herbinger et al., 1999). The use of genetic markers for assigning parentage and for pedigree analysis in "common garden" aquaculture experiments has become fairly common (O'Reilly et al., 1998; Fishback et al., 1999, 2002; Herbinger et al., 1999; Hara and Sekino, 2003; Sekino et al., 2003; Rodzen et al., 2004; Vandeputte et al., 2004) and allows evaluation of genotype × environment effects without confounding common environment effects. However, the high cost of molecular biology techniques necessary to carry out these analyses has limited the use of "genetic tagging" by commercial breeders. A true cost–benefit understanding of the genetic improvement made by reducing this common environmental effect weighed against the cost of the molecular analysis is greatly needed.

The specific objectives of this paper were to evaluate family growth response to fishmeal and gluten-based diets in a widely used commercial strain of rainbow trout which were previously selected for improved growth when fed standard fishmeal-based diets and to assess the magnitude of genotype × diet interactions in rainbow trout.

2. Materials and methods

2.1. Fish stocks

A commercial strain from Troutlodge, Inc. (Sumner, WA, USA), which has been under intensive, family-based selection program for improved growth for several generations provided the base population for this study.

2.2. Mating design and early rearing

Twenty individual females were mated to ten males in the following manner: two females were randomly assigned to be fertilized by a single male. This process was repeated for all ten males until gametes from each of the 20 females were fertilized. Gametes were collected on a single day and held at 4 °C until fertilization. Ovarian fluid was drained from the eggs, and 150 ml of a buffered saline solution (5 mM Tris, 2 mM glycine, 0.5% NaCl) was added along with approximately 5 ml of the appropriate milt. The egg/sperm mixture was gently mixed, allowed to rest for 5 min and then excess milt solution was decanted off. Eggs were water hardened in a 50 mg/l povidone iodine solution for 20 min. After this initial water hardening period, ambient water flow (10 °C) was reintroduced to the incubation tray and development proceeded until the "eyed" stage.

At eyed stage, an estimate of egg size was made using water displacement, and 1200 eggs from each full-sib family were retained. The eggs from all 20 families (24,000 in all) were pooled, mixed well, and randomly split into two groups. Each of these groups was incubated separately in a vertical tray incubator through hatching until the point of initiation of feeding.

At the initiation of feeding each group was placed into a separate rearing container of adequate size and supplied with first use ambient spring water (12 °C). Feeding was accomplished as described below, and bi-weekly sampling of average weight continued throughout the grow-out period of 294 days (approximately 350 g bodyweight). Fish on each diet were reared in three raceways. A schematic illustration of the experimental design is shown in Fig. 1.

2.3. Diet formulation, and feeding regime

The formulation of each diet is shown in Table 1. The starter, #1, #2 and #3 crumble sizes of each of the

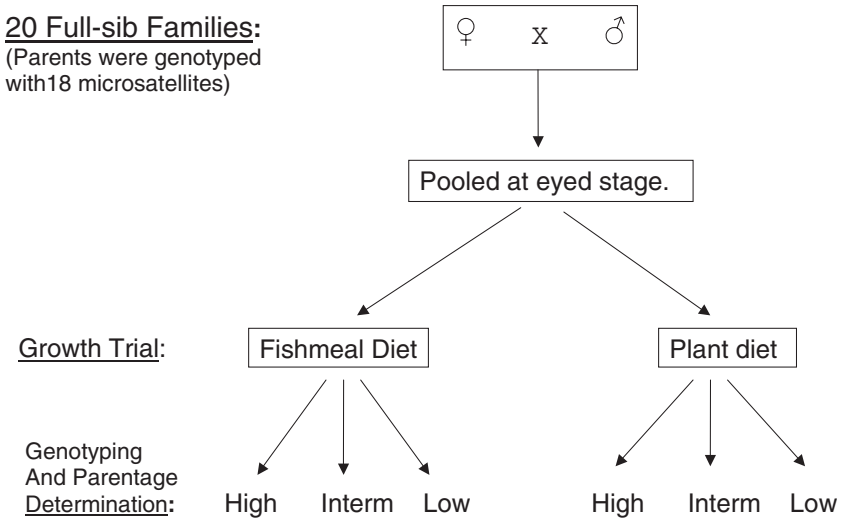

Fig. 1. Schematic representation of the 20 × 10 nested mating design, growth trial and parentage determination analysis.

experimental feeds were manufactured at the Feed and Nutrition Laboratory of the Fish Technology Center (Bozeman, Montana). These particles were produced using cold extrusion and dried using forced air of ambient temperature. All 2.0- and 3.0-mm feeds were produced in a commercial mill (Nelson and Sons, Murray, UT, USA). Krill meal was included in the gluten-based diet for the early rearing phases (through size #3 crumbles) to increase palatability. The krill meal was removed for the 2.0 and 3.0 mm feeds, leaving only the plant-based protein sources. For a period of approximately 2 months, 2.0-mm pellets produced by cooking extrusion were fed. The high level of wheat gluten in this feed was suspected of causing a very hard particle to be produced. Feed was then manufactured, for both diets for the rest of the study, by compression pelleting and feed consumption rate improved for the fish fed the gluten-based feed.

Table 1
Nutrient composition of pelleted diets fed to the two groups of rainbow trout

Plant diet		Fishmeal diet	
Ingredient	g/100 g	Ingredient	g/100 g
Corn gluten meal	33.42	Anchovy meal	40.54
Wheat gluten	20.67	Soybean meal	16.00
Krill	10.00	Blood meal	5.00
Wheat flour	13.77	Wheat flour	25.36
Fish oil	10.23	Fish oil	11.55
Lysine HCl	1.47	Vitamin premix #30[a]	0.60
Dicalcium phosphate	2.70	Choline chloride	0.60
Carophyl pink	0.05	Trace mineral premix #3[b]	0.10
Vitamin premix #30[a]	0.50	Carophyl pink	0.05
Soy lecithin	2.00	Stay-C	0.20
Trace mineral premix #3[b]	5.00		
Stay-C	0.20		
Total	100.0	Total	100.0

[a] Contributed per kg of diet: vitamin A, 10 000 IU; vitamin D_3, 720 IU; vitamin E, 530 IU; vitamin B_{12}, 30 μg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; folacin, 13 mg; menadione sodium bisulfate 25 mg; biotin, 1 mg; niacin, 330 mg.

[b] Contributed in mg/kg diet: zinc, 100; manganese, 70; iron, 3; copper, 2; iodine, 1.

The swim-up fry were fed manually for 2 weeks at half-hour intervals throughout a 10-h day until feeding patterns were established. Then the tanks were shifted to feeding by semi-automated feeder. Feed amounts were calculated using a proprietary feeding program and were adjusted weekly based on biomass estimates.

2.4. Sampling for parentage analysis

A random sample of approximately 250 fish from each raceway (a total of approximately 750 fish for each diet group) was measured for weight and length to estimate the correlation between these traits. Fish from three raceways per diet were hand-sorted according to length into three groups from each diet as follow, the longest (upper 2% tail of distribution; $n=235$ and $n=200$ for the gluten and fishmeal diets, respectively), smallest (lower 2% tail; $n=200$) and 100 fish selected randomly from the middle of the distribution. Those selected were fin clipped for DNA extraction, genotype analysis and assignment to family. The fish from the plant protein feed (gluten) large group were marked by

pit-tags for later breeding, and length and weight measurements were recorded for each individual. Fin samples collected from each of the 30 parents and the selected progeny were stored in 100% ethanol until DNA extraction. DNA samples were extracted as previously described (Palti et al., 2002a).

2.5. Genotyping of parents and simulations to identify the most informative markers

Eighteen unlinked microsatellite markers (Nichols et al., 2003) were used to genotype the parents. The criteria for markers selection were at least 50% heterozygosity and at least 5 alleles in the rainbow trout population that was used for markers characterization by Rexroad et al. (2001, 2002) and Palti et al. (2002b). Primer sequences, optimum annealing temperatures, $MgCl_2$ concentrations and Genebank accession numbers are given for each marker in Table 2. Primers were obtained from a commercial source (forward primers labeled with FAM or HEX; Alpha DNA, Montreal, Quebec, Canada). PCR reactions (12 μl total volume) included 50 ng DNA, 1.5–2.0 mM $MgCl_2$, 1.0 μM of each primer, 200 μM dNTPs, 1× manufacturer's reaction buffer (Qiagen Hotstar *Taq* 10×PCR Buffer), and 0.5 unit Qiagen Hotstar *Taq* Polymerase (Qiagen, Valencia, CA, USA). Amplifications were conducted in an MJ Research DNA Engine thermal cycler model PTC 200 (MJ Research, Waltham, MA, USA) as follows: an initial denaturation at 95 °C for 15 min, 30 cycles consisting of 94 °C for 60 s, annealing temperature of 45 s, 72 °C extension for 45 s; followed by a final extension of 72 °C for 10 min. PCR amplification was verified on 3% agarose gels. PCR reactions were then combined according to label and expected size for fragment size analysis with an ABI 3100 genetic analyzer and the GeneMapper software (ABI, Foster City, CA, USA).

The CERVUS program (Slate et al., 2000; http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html) was used to identify the markers most informative for parentage determination and determine how many markers will be used for progeny genotyping. The markers were ranked according to their polymorphic information content (PIC) value (Botstein et al., 1980). The error rate for parentage determination using CERVUS was estimated by inputting 20 virtual progeny genotypes for each full-sib family based on the known parents' genotypes and assumed Mendelian segregation. The first round of simulation included the 5 most informative markers, and each successive round included the markers from the last round and the next most informative marker up to a total of 18 markers. The program output of parentage assignments for each round of simulations was compared to the virtual families to estimate the error rate for that number of markers.

2.6. Genotyping of progeny and parentage analysis

Ninety-five (approximately 1%; random sample of the previously mentioned 2% for except for the gluten-high group where the longest and heaviest 95 fish were genotyped) of the progeny from each of the 6 groups

Table 2
Microsatellite marker information

Marker	Forward primer sequence	Reverse primer sequence	AT (°C)	$MgCl_2$	GeneBank
OMM1026	GGCATCAATATGGACTT	TCAGTCAAAAGGGTGTAG	58	2.0	AF346683
0MM1037	GCGACTGGATTTAATACTGC	TCCTCTGACTGCCATTACATC	56	1.5	AF346687
OMM1046	CAGGCACTATAATGGCAC	GCCCACGAGTTACAAGA	60	2.0	AF346693
0MM1012	TGCACTTCCGCTTCT	ATAGGACAGGGTAATGGG	60	2.0	AF346673
OMM1009	ACTGGAATCCAATAACAACCC	CGGAGGTTTGATGAGTCATT	58	2.0	AF346671
OMM1121	CTGCCAATGTTTGTCTATG	GGCTGTCAATCTGTCTTCTAC	62	1.5	AF375027
OMM1017	GCCATTTCCGTTCCAATTT	AAGGTCAGAGAGGTAGCCAAG	62	2.0	AF375009
OMM1148	TGGCTTGACTCCTACCCCAGTCTATT	AGAGAAGAGGTGGAAAGAAAGAGTGCG	58	2.0	AY039630
OMM1051	CCTACAGTAGGGATTAACAGC	CATGCCCACACATTACTAC	58	2.0	AF346695
OMM1101	CTGCCTCTGATTGAGAACCATATC	CCGTGTCAGATGAATTGGG	62	2.0	AF352765
OMM1117	AAGCCAGAGGGGATAAGATG	GCAATGGGCTCTATGACTGAT	62	2.0	AF352771
OMM1025	CGCCATTGTAGTCTCGTC	AGTCCGCTATGTTGTTATGTC	58	2.0	AF346682
OMM1134	GAAGTTCATCTCCAGGTCAAACTG	TGCGTAGGTTGATGAATCCTC	58	2.0	AY039628
OMM1045	TTGCCTGTGATGACTGGACTCTAT	GCAGGTGTCTCCATAACAACGA	60	2.0	AF346692
OMM1015	GACAAATTCACCCTCTTATG	CATGAGAACTGTTGCCA	58	2.0	AF346675
OMM1039	GGGGTAGGAGTAGACTAGACA	ATCTTTCCCTCCTTGCAC	56	2.0	AF346689
OMM1019	CCAGCAGTAAACCTTAGGTG	GTCAAAGGAGACGTAGAGCTT	58	2.0	AF346678
OMM3027	GCTGCTTCCTGTATGTACTCT	TCAGCTGGAGGTACTTTAA	62	2.0	BV012586

described above (Section 2.4) were genotyped with the 6 most informative markers (*OMM1025*, *1026*, *1046*, *1051*, *1101*, *1148*) and the parents and progeny genotypes were used as input for parentage determination with CERVUS. Progeny that were assigned by CERVUS to parents that were not mated were assigned manually to actual full sib families. The parentage assignment program PAPA (Duchesne et al., 2002; http://www.bio.ulaval.ca/louisbernatchez/downloads.htm) was used to retest the results and compare the findings obtained by the two parentage programs.

2.7. Statistical analyses

Estimates of correlations between length and weight based on individual ranking and the distribution of each trait in each diet were obtained using Analyse-it software package, descriptive statistics and ANOVA modules (Analyse-it Software, Ltd.).

Sires were each mated to two females which permitted nested analysis of sire effects. Therefore analysis of the family frequency is based on appearance by sire grouping in the large (high growth), small (low growth)or intermediate growth groups and the interaction between sire and diet was conducted using a maximum likelihood SAS statistical system procedure for categorical data modeling, Proc LOGISTIC, to fit a proportional odds model (Stokes et al., 2000). The overall significance of the male genotype and of diet effects on family frequency were examined as well as the odds of each male to be represented in the high growth category. Contrast statements were analyzed to determine the significance of the growth differences among the 10 sires.

The underlying model was defined as:

$$\mathrm{Log}\left(p_{ij1}/\left(p_{ij2}+p_{ij3}\right)\right) = a + x_{ij}^{*}b,$$

where p_{ijk}, $k=1, 2, 3$, represents the frequency in the high, intermediate, and low growth group, respectively, x_{ij} represents an indicator variable (i.e., 0 or 1) for the ijth treatment combination ($i=1, 2$ for the diet and $j=1, 2, 3, \ldots, 10$ for the sires), and a and b represent a slope and a vector of treatment effects: $b=[b1, b2, b3, \ldots, b10, b11, b12]$ (where $b1, \ldots, b10$ correspond to the 10 sires and $b11$ and $b12$ correspond to the two diets).

To evaluate the equality in survival rates among the different families, a chi square goodness of fit test with correction for continuity was employed (Zar, 1984). Equal representation among the 20 families was hypothesized in the gluten diet and fishmeal diet intermediate groups.

3. Results

The fish were grown for 294 days at which point they reached an average weight of 358 g and length of 296 mm in the fishmeal diet and 397 g and 304 mm in the gluten diet. Survival during the feeding trial was 95.0% and 94.6% on the fishmeal and gluten diets, respectively. While length and weight of the random samples from each diet group were significantly correlated ($P<0.001$), length and weight were not correlated ($R^2=0.20$) in the gluten-high group ($n=235$).

Error rates for parentage determination with the 5 and 6 most informative markers in the CERVUS simulation were 17.5% and 7.5%, respectively. Error rates in parentage determination with up to 18 markers and the corresponding markers' PIC values are listed in Table 3. Only 5 markers were used in the final parentage analysis since one of the markers (*OMM1025*) is likely to contain null alleles (Palti and Rexroad, unpublished data).

Representation of full sib families in the high, intermediate and low sub-groups from each diet as determined by both the CERVUS and PAPA programs is shown in Table 4. Approximately 11% of the progeny (62) were assigned by CERVUS to parents that were not mated. Sixty of those progeny were successfully assigned to full-sib families by manually matching their genotypes and the genotypes of the parents. Approximately 91% of the progeny were assigned to parents using the "no error" model for PAPA analysis. However, since this program allows using models that consider genotyping errors and mutations we applied the uniform error model (using the program's default settings), which enabled parentage assignment for all the genotyped progeny (Table 4). The PAPA assignments were nearly identical to those we obtained using the compounded CERVUS analysis and manual corrections.

No interaction was identified between genotype and diet as the ranking of the half-sib families was very similar for both diets. The sire effect was significant ($P<0.001$). The dam effect could not be estimated due

Table 3
Error rates in parentage determination using CERVUS and range of markers PIC values

Number of markers	Error rate (%)	Range of PIC values
All 18	3.75	0.684–0.885
Top 10	5.00	0.773–0.885
Top 8	6.25	0.791–0.885
Top 6	7.50	0.814–0.885
Top 5	17.50	0.829–0.885

Table 4
Family representation in the high and low "tails" and in the intermediate group from each diet as calculated from the CERVUS software analysis

Family	Parents	GL[a]	GI[a]	GH[a]	FML[a]	FMI[a]	FMH[a]	Total
1	1M×1F	18 (18)	5 (5)	0 (0)	18 (18)	5 (5)	1 (1)	47 (47)
2	1M×3F	19 (19)	6 (6)	0 (0)	12 (12)	2 (2)	1 (1)	40 (40)
3	3M×8F	1 (1)	5 (5)	9 (9)	0 (0)	12 (12)	31 (31)	58 (58)
4	3M×9F	0 (0)	3 (3)	2 (2)	1 (1)	2 (1)	8 (8)	16 (15)
5	5M×13F	6 (6)	6 (6)	0 (0)	8 (8)	3 (4)	0 (0)	23 (24)
6	5M×14F	0 (0)	3 (3)	0 (0)	0 (0)	5 (5)	3 (3)	11 (11)
7	6M×16F	1 (1)	8 (8)	41 (41)	1 (1)	4 (4)	15 (15)	60 (60)
8	6M×17F	0 (0)	3 (4)	30 (30)	1 (1)	10 (9)	20 (20)	64 (64)
9	7M×19F	0 (0)	6 (6)	0 (0)	1 (1)	3 (4)	0 (0)	10 (11)
10	7M×21F	3 (3)	3 (3)	9 (9)	9 (9)	6 (6)	3 (3)	33 (33)
11	8M×22F	2 (2)	5 (5)	0 (0)	0 (0)	8 (8)	0 (0)	15 (15)[1]
12	8M×23F	10 (10)	0 (0)	0 (0)	16 (16)	2 (2)	0 (0)	28 (28)
13	9M×26F	2 (2)	5 (5)	2 (2)	5 (5)	4 (5)	3 (3)	21 (22)
14	9M×27F	1 (1)	5 (5)	2 (2)	0 (0)	6 (6)	6 (6)	20 (20)
15	10M×29F	6 (6)	7 (7)	0 (0)	8 (8)	6 (6)	0 (0)	27 (27)
16	10M×30F	8 (8)	2 (2)	0 (0)	3 (3)	1 (1)	2 (2)	16 (16)
17	11M×31F	1 (1)	5 (5)	0 (0)	1 (1)	3 (2)	0 (0)	10 (9)
18	11M×32F	2 (2)	6 (6)	0 (0)	0 (0)	2 (3)	0 (0)	10 (11)
19	12M×34F	7 (7)	8 (8)	0 (0)	6 (6)	4 (4)	2 (2)	27 (27)
20	12M×36F	8 (8)	3 (3)	0 (0)	5 (5)	6 (6)	0 (0)	22 (22)
Not typed:[b]		0 (0)	1 (0)	0 (0)	0 (0)	1 (0)	0 (0)	2 (0)
Total:		95	95	95	95	95	95	570

The numbers in parenthesis are based on parentage analysis using the PAPA software. Differences between the two programs are highlighted in gray shade.
[a] GL = gluten diet low growth; GI = gluten intermediate; GH = gluten high growth; FML = fishmeal low; FMI = fishmeal intermediate; FMH = fishmeal high.
[b] Genotype determined for offspring did not match any of the possible genotype combinations from the parents.

to small sample size in some of the full-sib families. Pair-wise comparisons between the 10 half-sib families revealed major differences in the performance of individual sires (Table 5).

The chi-square goodness of fit test was significant ($P<0.05$) revealing that not all families were equally represented among the intermediate groups on both diets. More detailed examination showed that families 12 and 16, belonging within sires 8 and 10, respectively, were underrepresented (Table 4) and the primary cause of nonconformity with the expectation of equal representation among families.

Table 5
A summary of pair-wise comparisons between the 10 half-sib families

Sire	OR[a]	Log (OR)	Letter groups[b]
6	170.7	5.14	a
3	96.5	4.57	b
9	18.2	2.90	c
7	11.5	2.44	cd
11	7.3	1.99	cde
5	4.8	1.56	def
12	2.9	1.05	efg
10	2.4	0.87	fg
8	1.7	0.54	gh
1	1.0	0	h

[a] OR = odds ratio (e.g., offspring from sire 6 are 170.7 times more likely to be classified as heavy than offspring from sire 1).
[b] Any two ORs with no letter in common imply that the two odds are significantly different ($P<0.05$) by use of Wald's χ^2 test.

4. Discussion

A significant genotype × diet interaction was not observed in this study, which suggests that fish that grow faster when fed fishmeal diets are likely to grow faster when fed the particular gluten-based feed used in this study. We found large genetic variation and highly significant sire effect on growth in the commercial strain that was used in this study, which suggests that additional gains can be achieved by selection for large body size. Our findings are in agreement with the results of Blanc (2002), who concluded that "genetic improvement of growth should suffer little impairment from possible changes in future feed formulations".

The plant protein-based diet we used was selected because it was known to support a high rate of weight gain. Gluten meals are protein concentrates that have many of the anti-nutrients (i.e. poorly digestible carbohydrates) present in plant-derived ingredients removed. The high wheat gluten levels in this feed may also have

a negative effect on pellet quality when produced by cooking extrusion. New methods of concentrating protein and removing anti-nutrients are being developed; these will decrease the cost of fishmeal free feeds in the future.

The overall high survival rate, and the even representation of all families in the intermediate groups, with the exception of families 12 and 16, and the good representation of all families in the sum of all groups (at least 10 fish per full-sib family; Table 4) suggest that family-based differences in survival did not skew the likelihood to appear in the high or low groups. Therefore, the over-representation of progeny from sires 3, 6 and 9 in the high groups and on the other hand over-representation of progeny from sires 1, 8 and 10 in the low groups are likely to be the result of genetic affects on growth. The only difference in family ranking between diets was in the two top sires, sires 3 and 6. While both performed equally well when fed fishmeal diet, sire 6 was significantly ($P<0.05$) over-represented in the gluten-high group. This suggests that interaction may exist at the extreme high end of the performance distribution and a study with a larger number of families should be conducted to further investigate and identify this putative genotype × diet interaction.

The low correlation we observed between weight and length in the gluten-high group is somewhat surprising, as these two traits were found to be highly correlated in our random pre-sampling of the population and in previous studies (e.g. Silverstien and Hershberger, 1995). In addition to sampling the gluten-high group by length (family distribution of longest 95 fish is described in Table 4), we sampled and genotyped the heaviest 91 fish. The gluten high by length sub-group contained 38 fish that were not present in the gluten high by weight section, while 35 of the weight section were not included in the length section. The only difference in family ranking was that the over-representation of sire 6 progeny in the length sub-sample was less pronounced, and practically equal to that of sire 3 in the weight group (data not shown). The ranking of the other families remained the same.

Based on the parental genotypes and simulations with CERVUS, we identified 6 markers that were expected to assign 92.5% of the progeny correctly. However, progeny genotyping revealed that one of the 6 markers (*OMM1025*) was not informative for parentage assignment. This is likely due to the high occurrence of null alleles in this marker (Palti and Rexroad, unpublished data). The expected error rate for CERVUS parentage assignment with the other 5 markers was 17.5%. However, since 11% of the progeny were assigned incorrectly by CERVUS to parents that were not mated, and then re-assigned correctly manually, we assume that the actual error rate in parentage assignment here was 6.5%. This rate is not likely to have any impact on the validity of our highly significant results. To re-evaluate our pedigree assignment, we also analyzed the genotypes with the PAPA software that applies allelic exclusion algorithm, and therefore should be more suitable for analyzing closed population structure with known crosses. As shown in Table 4, the pedigree assignments by both methods were nearly identical. However, for future work with similar populations we would strongly recommend using allelic exclusion software like PAPA that eliminated the manual corrections step in this study. This software also indicates where the genotypic variation is not sufficient to assign pedigrees and accommodates certain levels of mis-match or mis-scoring data, and therefore reduces the error rate in parentage assignments.

To further improve the efficiency and to lower the cost of pedigree assignments in large-scale commercial breeding programs, the development of a set of microsatellite markers that can be designed to combine according to label and size for fragment analysis and processed in a single PCR reaction (multiplexing) would be very useful.

An interesting observation that was revealed by genotyping was the high occurrence (35 of 47) of triploids among the progeny of sire 1 and female 1. We deemed an individual triploid if at least 2 of the 5 markers we used had 3 alleles. Therefore, it is possible that the actual percent of triploids among progeny from this cross was even higher. The parents of triploids were identified by matching 2 alleles to one parent and one to the other parent. Markers with 3 alleles were found in fish that could only be assigned to sire 1 and dam 1, and the source for the 2 alleles in all of them was the dam. The occurrence of increased rate of spontaneous second polar body retention in some rainbow trout females was previously reported (Palti et al., 1997). However, rather than being controlled by genetic factors, this phenomenon may be associated with increased post-ovulatory oocyte aging as suggested by Aegerter and Jalabert (2004). Reduced performance of triploids in mixed culture with full-sib diploids was previously observed (Blanc et al., 2001), which may partially explain the poor growth performance of the progeny from this cross. However, it is important to note that the performance of the all-diploid progeny from the half-sib family of sire 1 and dam 3 was equally poor, which indicates that there was also a strong sire effect involved.

In conclusion, we found that commercial strains of rainbow trout, which were previously selected for improved growth when fed standard fishmeal-based diets, should retain their improved growth performance if fed a gluten-based diet; however, the potential of genotype × diet interactions in other populations, and with less purified plant-based feeds, should be further investigated. Additionally, the effects of plant protein-based diets on reproductive success, disease resistance and other production traits should be assessed.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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